Sequential [^{125}I]-o-Iodohippurate Renograms from Rabbit Kidneys after Temporary Renal Ischemia

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Serial renography with [^{125}I]-o-iodohippurate was performed in 42 rabbits after temporary clamping of the renal artery for 1–3 hr and contralateral nephrectomy. Renograms were done before and after warm ischemia and followed 2, 4, 6, and 14 days after restoration of circulation. Correlation was found between duration of ischemia and severity of postischemic renographic changes. Most sensitive was the third phase, which showed distortion after 1 hr of ischemia; the second phase decreased after 2½ hours of ischemia. The profound changes seen on the second day were shown to be due to further damage of the tubular cell system after restored flow. In vitro [^{125}I]-o-iodohippurate uptake in cortex slices showed a significant decrease after warm ischemia damage and 48 hours restored flow. The second phase recovered more rapidly than the excretory function.

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Renography provides suitable monitoring of the transplanted kidney. Information on renal blood flow, tubular function, and urine excretion is gained, and minor disturbances are detected. It is a sensitive method, but it lacks specificity because numerous competitive factors cause similar changes in the renograms.

In experimental models, controlled studies of any single factor can be made. In the present study the influence of warm ischemia on the renogram and kidney function under standardized conditions is investigated.

MATERIAL AND METHODS

Female rabbits of Danish landbreed, with an average weight of 2.7 kg, were used in 42 experiments. The animals were anesthetized with Nembutal (R) 8–12 mg/kg. The blood pressure and abdominal temperature were registered and controlled during anesthesia. Preoperatively, isotonic NaCl (15 ml/kg) and heparin (500 IU/kg) were given intravenously. Oxygen and fluid supplements were given during the operation. Anesthesia was maintained with Nembutal, 2–4 mg/kg, every hour. The abdomen was opened in the midline and the kidney approached transperitoneally. The right kidney was removed, and a renogram of the left kidney performed. The left renal artery was then clamped for 0, 1, 1½, 2, 2½, or 3 hr. The duration of ischemia in each experiment was allocated at random. Five minutes after renal circulation was restored, another renogram was performed. The abdomen was then closed. In the control group the right kidney was removed, the left not touched, and the rabbit kept in anesthesia for 3 hr.

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Postoperatively the animals were allowed to drink freely. They were weighed daily and isotonic glucose supplements given, if weight loss was in excess of 50 g/day.

Renographic technique. [^{125}I]-o-iodohippurate, 4.4 μCi, was injected as a bolus intravenously in a cannulated ear vein. A scintillation detector with a ¼- × ¼- × ¼-in. NaI crystal in a lead collimator of kidney size was used. The detector was placed directly over the kidney during the operation. The renograms in the days following surgery were performed with the rabbit lying on its back in light Nembutal anesthesia. The kidney was easily palpated with the animal supine, and the detector was placed directly above the kidney with only a thin layer of skin and abdominal muscle interposed. All renograms were followed for 20 min. Renograms of 62 normal anesthetized animals were investigated and compared with renograms of ten normal nonanesthetized rabbits.

Renography was performed before and after arterial clamping, and 2, 4, 6, and 14 days after reestablishment of circulation.

Three phases were used to evaluate the renograms: the initial fast rise in activity (first phase), the uptake or second phase, and, if the curve passed through a peak, an excretory or third phase. Steadily rising activity was called an accumulation curve (*4*). The renograms were processed by means of a light pen computer. After background subtraction, the first phase, time to maximum activity, and the activity of the curve every minute after the iodohippurate injection were calculated. From these values "mean" renograms for each specific group and specific day were calculated as means and s.d. of the values. For evaluation of the second phase, the mean activity after one min was measured. An excretory or uptake ratio, defined as the 10-min to 20-min activity, was calculated. The groups were compared using Student's t-test.

Meticulous care was taken to avoid changes of the renogram due to technical errors—e.g., twisting of the kidney by pressure from a detector placed too close to the kidney, dehydration, too deep anesthesia, or low arterial blood pressure. These pitfalls can often be reflected in a prolonged excretory phase.

Repeated investigation of the same animal showed a variation of ±5% from the mean values of the renograms taken within 1 hr.

Iodohippurate uptake in slices of renal cortex. This technique has been described in detail in a previous article (*2*). The iodohippurate uptake was measured using a modification of the method described by Cross & Taggart (*3*). The cortex was sliced manually with an ice-cooled microtome. The 0.2-mm slices were distributed evenly among eight incubation vessels. Each vessel contained one μCi of iodohippurate in 50 ml Cross-Taggart solution. The solution was oxygenated before and throughout the following incubation for 60 min at +25°C. The vessels were then rapidly cooled to +4°C and the slices removed and weighed. The slices, and also aliquots of 1 ml of the incubation fluid, were counted for 60 sec in a gamma scintillation counter. The [^{125}I]-o-iodohippurate uptake was calculated as the ratio between the iodohippurate content per gram of tissue and that per milliliter of incubation fluid—i.e. the slice-to-medium ratio. Iodohippurate uptake was measured in kidneys exposed to warm ischemia of 1 and 2 hours' duration and either removed immediately for measurement or reperfused for 48 hr before removal. Each left kidney, which had been clamped, was compared with the right kidney, which was untouched, providing an individual control.

RESULTS

Renography. Figure 1 shows the averaged renograms for the different groups, (a) before and 5 min after arterial clamping of stated duration, and (b) the repeat renograms on the 2nd, 4th, 6th, and 14th days after the operation. The renograms of kidneys exposed to 3½ hours of anesthesia and the surgical trauma of removing the contralateral kidney were mainly unaffected, compared with normal kidneys of animals investigated without or during anesthesia. In this group, slight, nonsignificant decreases in the first and second phases were seen on Days 0 and 2. The renograms after 1 hr of ischemia showed a steady iodohippurate accumulation for 15 min before a maximum was reached, and the drainage phase was very slow. On the second day there was a slight decrease in the uptake phase, with a drainage phase that was normal although delayed. The renograms were normal again on the 4th day after the operation. More pronounced changes were seen after 1½ hr of ischemia. The uptake phase was slower, but a maximum was reached after 9 min. On the second day a 50% decrease in the uptake phase was seen. The maximum appeared late and was followed by a slow drainage phase. The renogram was normal again on the 4th day. The renograms following 2 hours of anoxia showed reductions in the uptake phase and a retention type of curve. Even more pronounced decreases in uptake were seen on the second day and on the 4th and 6th days, but in the latter cases delayed drainage phases ensued. The maximum was reached within normal time on the 6th day, but on the 14th day

showed a delayed time to maximum with normal appearance and uptake phases.

Not all animals survived 2½ and 3 hr of warm renal ischemia. Slow appearance and uptake phases were seen in both groups following the ischemia, and no drainage phases appeared. On the second day after the operation the uptake was very weak; later the renograms in the group with 2½ hr of ischemia showed greatly decreased uptake and delayed drainage, whereas the renograms after 3 hr of ischemia remained nearly flat.

Figure 2 depicts the uptake phase in terms of the 1-min activity for all the groups. These values were taken as an index of the combined renal blood flow and tubular activity. No significant differences were seen in the controls following the sham operations, even though the long anesthesia influenced the uptake. Characteristically, a fall in activity was seen

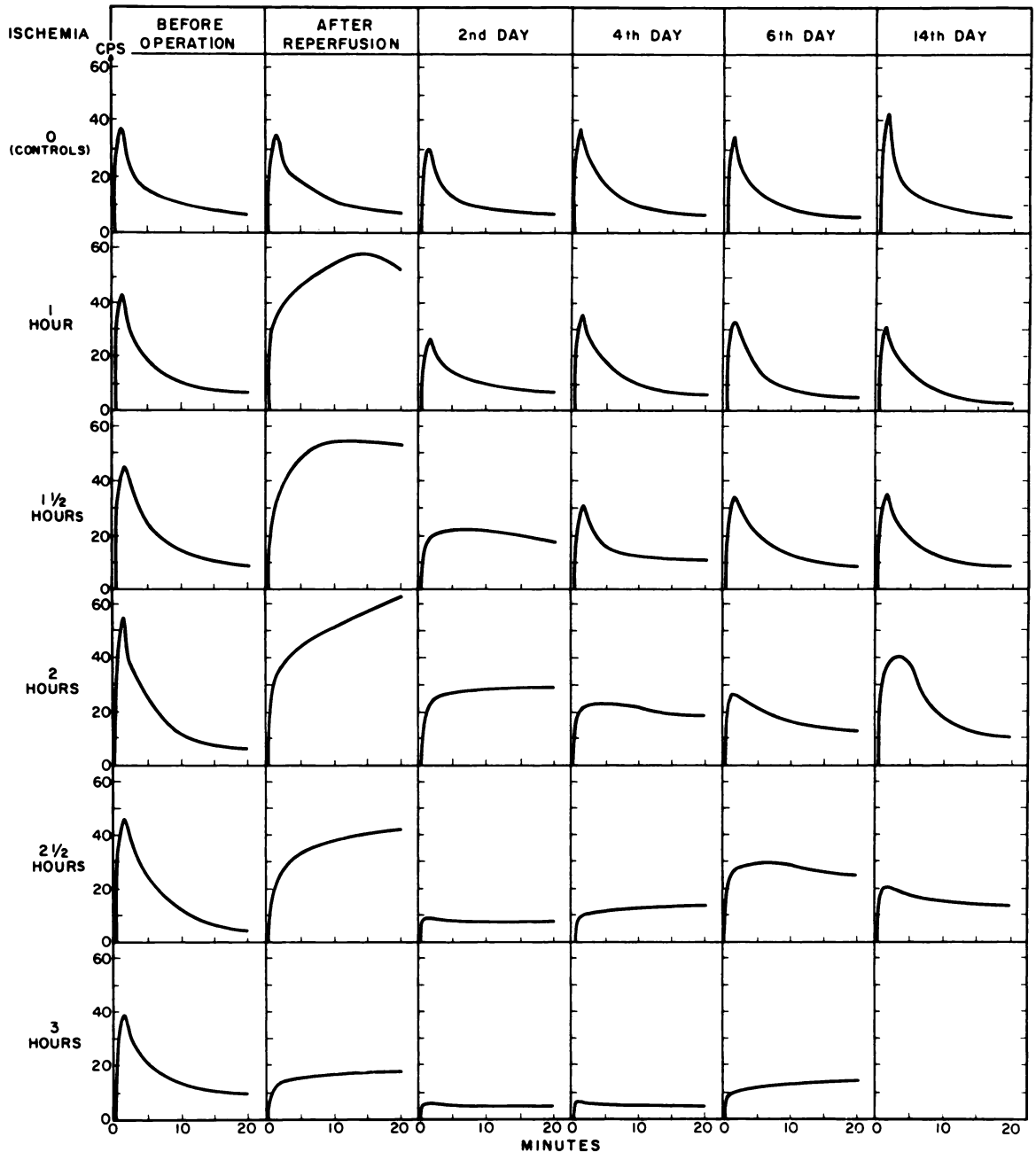

FIG. 1. [^{125}I]-o-iodohippurate renogram for the groups of rabbits exposed to warm ischemia for 0, 1, 1½, 2, 2½, and 3 hr. Mean renograms are derived for different groups before and after arterial clamping and on 2nd, 4th, 6th, and 14th days after circulation was restored.

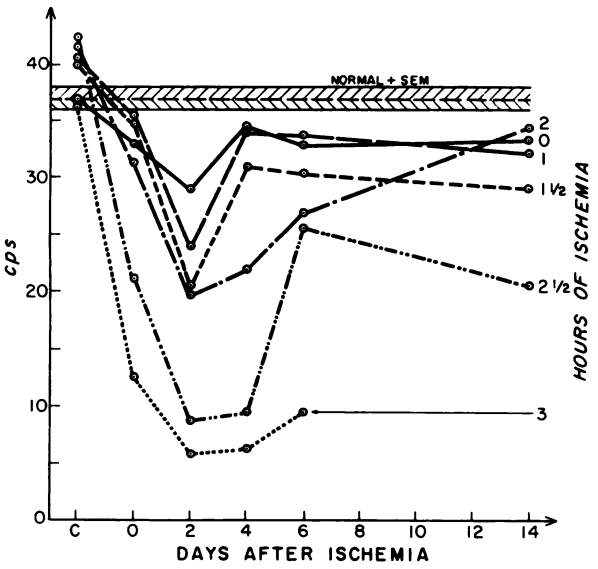

FIG. 2. One-minute activity in uptake phase of renograms of kidneys exposed to arterial occlusion for 0, 1, 1½, 2, 2½, and 3 hr. Values from mean renograms of the groups before occlusion (C, in abscissa), after restored circulation (0) and after 2, 4, 6, and 14 days.

after the ischemia in all groups—more pronounced the longer the duration of ischemia. There was, however, a remarkable further deterioration of the uptake seen in all groups on the second day after the operation. At this time the uptake had fallen to 50% after 1½ hours of anoxia, and to 16% after 3 hr. Some recovery was seen in the following days for all groups, but none of them regained normal values within 14 days.

Table 1 illustrates the correlation between the duration of ischemia and time to maximum. The more prolonged the ischemia, the longer the time to maximum.

The ratios between the activities at 10 and 20 min are seen in Fig. 3. The sham-operated control group is omitted from this figure, since it showed no deviation from the normal. The excretion ratio is below normal for the remaining groups after reperfusion. The normal ratio was seen on the second day for 1 hr of ischemia and on the 6th day after 2 hr, but never after more protracted periods of ischemia.

Iodohippurate uptake in cortex slices. In an earlier investigation (*2*), the iodohippurate uptake, measured as slice-to-medium ratio in normal cortex slices was found to be 19.3 ± 2.5. Decreases to 17.2 ± 2.5 and 16.4 ± 5.0 were seen after 1 and 2 hr, respectively, of warm ischemia. For the present work, Table 2 shows the iodohippurate uptakes, measured 2 days postoperatively, for slices from the left (ischemia-damaged) kidney and the undamaged contralateral control. After 1 hr of ischemia the uptake was 9.9 ± 1.7, equivalent to 73.7 ± 8.2% of control. Two hours of ischemia gave a decrease to 5.4 ± 4.5, equal to 36.0 ± 27.5% of control. This difference between 1 and 2 hours ischemia is significant ($p < 0.01$).

Figure 4 shows the correlation between the second phase of the renogram (in percentage of the preischemic value) and the in vitro uptake in cortex slices. The groups with 1 and 2 hours of arterial occlusion showed a decrease to 88 and 75%, respectively, immediately after reperfusion. After 48 hours of restored flow, further decreases in uptake were seen to 58 and 48%, respectively. The decreased uptake in cortical slices is seen to be of the same order of magnitude.

Serum creatinine. Changes in serum creatinine are illustrated in Fig. 5. The preoperative values and those on the 2nd, 4th, 6th, and 14th days are shown. No significant rise was seen after 1 or 1½ hr of clamping. After 2 hr of clamping, a significant increase was seen on the second day ($p < 0.05$), but some recovery is evident by the 4th day. After 2½ or 3 hr of clamping, significant rises to 0.78 millimols/l ($p < 0.001$) and 1.14 millimols/l ($p < 0.001$), respectively, were seen on the second day. After 2½ hr of ischemia, the serum creatinine had not recovered on the 14th day and it remained per-

TABLE 1. RABBIT KIDNEYS EXPOSED TO WARM ISCHEMIA: TIME (MIN) FROM INJECTION OF [^{125}I]-o-IODOHIPPURATE TO RENOGRAM PEAK (MEAN ± S.D.)

	Duration of warm ischemia (hr)					
	0	1	1½	2	2½	3
	Time to renogram peak (min ± s.d.)					
Before clamping	1.1 ± 0.3	1.4 ± 0.5	1.8 ± 0.6	2.7 ± 1.2	1.8 ± 0.6	1.5 ± 0.4
Clamp released	1.5 ± 0.9	10.0 ± 6.0	3.1 ± 4.7	> 20	> 20	> 20
Days after arterial clamping						
2	1.2 ± 0.3	1.3 ± 0.6	6.2 ± 5.3	> 20	> 20	> 20
4	1.5 ± 0.5	1.5 ± 0.5	1.3 ± 0.3	2.0 ± 2.0	> 20	> 20
6	1.2 ± 0.4	1.6 ± 0.2	1.5 ± 0.4	2.3 ± 2.3	7.3	> 20
14	1.4 ± 0.5	0.9 ± 0.1	1.9	2.5 ± 1.7	1.3 ± 1.3	> 20

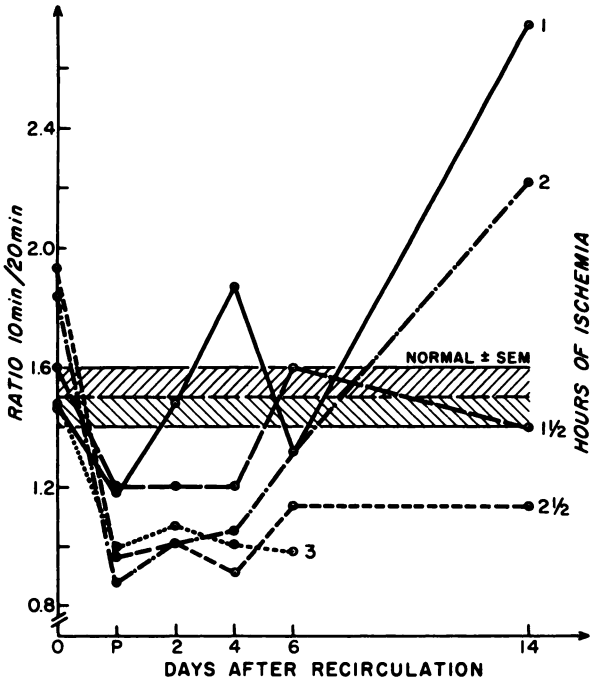

FIG. 3. Excretion ratio, $\frac{\text{ordinate at 10 min}}{\text{" " 20 "}}$, obtained from mean renograms of groups exposed to temporary warm ischemia, taken before (0) and after renal arterial occlusion (P), and at 2, 4, 6, and 14 days after restored circulation.

sistently high in the group with 3 hr of ischemia.

Survival. All animals lived after 2 hr or less of renal ischemia; four out of ten tolerated 2 hr; but only one of ten survived after 3 hr of clamped renal artery.

DISCUSSION

The aim of the present investigation was to illustrate with successive renograms the consequences of warm ischemic damage to kidney function and its restitution.

To minimize other factors influencing the renograms, the experimental conditions were highly standardized. Right-sided nephrectomy was performed on animals of the same sex, race, and weight. Temperature, blood pressure, and hydration were kept under control. The animals were hydrated every day. Ischemia was produced by a clamp on the left renal artery, and renograms were taken before and after clamping and followed for 14 days. The duration of ischemia was allocated at random for each animal.

It has been shown in an earlier study (*4*) that clamping of the renal artery results in ischemic damage comparable to the anoxic trauma observed in the human cadaver kidney. No attempt was made to eliminate the collateral circulation, found earlier to be without importance in rabbits (*5*); neither were the kidneys denervated, as no difference between intact and denervated organs has been demonstrated (*6*).

The renograms were evaluated by the few simple parameters most often used for evaluation of renograms (*1, 7, 8*). The 1-min value of the second phase is indicative of the renal blood flow and tubular function. The excretion ratio is dependent on the amount of iodohippurate taken up by the kidney, as well as the amount leaving the kidney in the same time interval; it gives information on glomerular filtration, nephron flow rate, and diuresis (*9*).

Our results show that rabbit kidneys tolerate up to 2 hr of ischemia without long-lasting sequelae, whereas kidneys exposed to 2½ and 3 hr of ischemia never regain normal function. A comparison with other clamping experiments, considering differences in species, survival rate, and histologic changes, has been reported elsewhere (*10*).

TABLE 2. [^{125}I]-o-IODOHIPPURATE UPTAKE IN SLICES OF CORTEX FROM LEFT KIDNEYS EXPOSED TO 1 OR 2 HR OF WARM ISCHEMIA (RENAL ARTERY CLAMPED), FOLLOWED BY 48 HR OF RESTORED CIRCULATION*

	48 hr of restored circulation preceded by:			
	Right control	Left Warm ischemia 1 hr	Right control	Left Warm ischemia 2 hr
Slice:medium ratios	10.5	6.4	11.8	1.2
	14.6	10.9	12.8	6.5
	13.5	10.6	13.9	3.7
	12.4	9.8	15.6	1.2
	16.2	11.5	21.0	5.4
	13.4	10.0	8.9	1.4
	13.0	11.3	15.4	10.5
	13.8	8.7	17.8	13.3
	Mean ± s.d.		Mean ± s.d.	
	13.4 ± 1.6	9.9 ± 1.7	14.7 ± 3.7	5.4 ± 4.5

*Values for $\frac{\text{I-125/g in slice}}{\text{I-125/cc in surrounding fluid}}$ are compared with those for normal contralateral kidney.

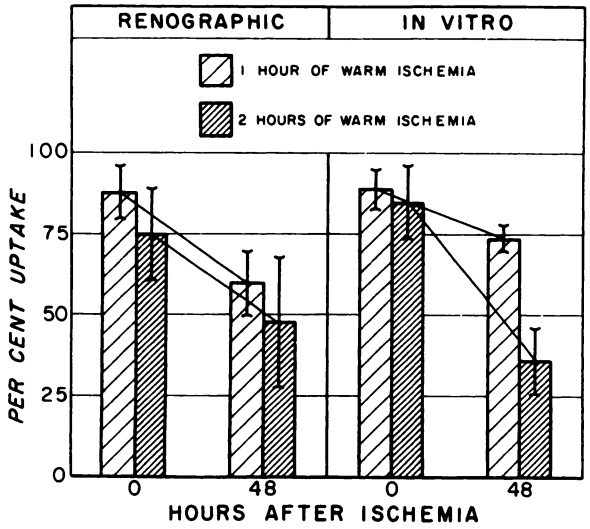

FIG. 4. Comparisons between uptake phase of renogram (as percentage of the preischemic value) and in vitro uptake of Hippuran in cortex slices, after exposure to temporary renal arterial occlusion and after 48 hr of restored circulation.

The renograms taken immediately after renewal of the circulation showed that ischemia of shorter duration affects primarily the third phase, resulting in late maximum peak and delayed excretion. Protracted ischemia affected the second phase also.

On the second day, the 1-min uptake was lower than after reperfusion in all the groups apart from the control group. This decrease was more pronounced the longer the duration of arterial clamping.

On the 4th and 6th days, the renograms showed improvements in uptake, but slower restitution was seen in the excretory phase. The damage to kidney function, as judged by renogram, was proportional to the length of ischemia; recovery was proportionately slow.

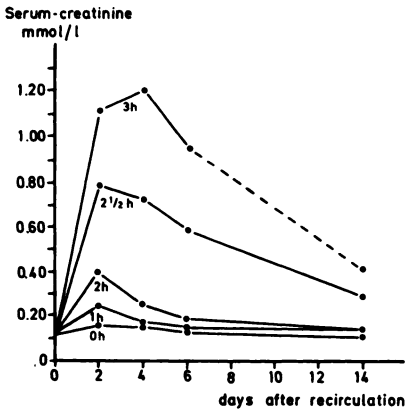

FIG. 5. Time course of serum creatinine in groups after temporary renal arterial occlusion for 0, 1, 2, 2½, and 3 hr.

It is remarkable that the renograms on the second day showed more pronounced changes than the postischemic renograms. One would have expected that both blood flow and tubular function would gradually improve after the damage.

The reduced-uptake phase must be due either to a reduced blood flow and/or to a decreased tubular-cell ability to secrete iodohippurate.

It has been shown by several investigators, using techniques not dependent on tubular function, that renal blood flow after arterial clamping is nearly normal after reperfusion (*11-14*), and most investigators describe return to normal flow within 3-7 days. Studies of the microcirculation likewise reveal nearly normalized flow after 48 hr (*15*). There is not full agreement among the investigators, however, as others find reduced cortical flow 24-48 hr after 2 hr of arterial occlusion (*16-20*). Hyperemia immediately after renal arterial occlusion has not been observed in the above-mentioned investigations; thus it is not likely that marked hyperemia would compensate for decreased cell activity by carrying more iodohippurate to the kidney on the day after arterial clamping. A decrease in blood flow on the second day is also unlikely as an explanation of the more reduced iodohippurate uptake.

To test whether the tubular ability to excrete iodohippurate is further reduced on the second day after ischemic damage, we investigated the iodohippurate uptake in isolated cortex slices and found a parallel decrease in this uptake, as seen in the 1-minute uptake of the renograms after 48 hr of restored circulation. These results are in accordance with the findings of Reimer and Jennings (*21*) on the progressive disintegration of tubular function as the duration of restored flow was extended to 24 hr. Advanced necrotic changes of the tubular cells have been seen histologically after restored flow (*20, 22-24*). Repair processes are usually seen from the third day. Histochemical investigations show signs of repair on the second day (*15*). PAH and inulin clearance are grossly diminished after ischemic damage (*19, 25*), but improvements can be seen on the first day after restored flow (*11, 26*).

This observation may be helpful in avoiding the pitfall of diagnosing rejection crisis in the transplanted kidney, since it produces a similar renographic picture (*27-29*).

The restitution of the renograms and kidney function was fair up to 2 hr of ischemia. The postischemic renogram predicted survival and recovery of kidney function if the 1-min iodohippurate uptake was within normal limits. A horizontal curve on the second day may warn of renal failure and death in uremia, but several animals regained uptake and

function. Therefore, predictive value of the renogram on this day was not accurate.

Recovery of the renograms began with an improvement in the uptake phase, then a late maximum peak was seen, followed by a torpid excretion phase. We cannot confirm the findings of Lundgren et al. (*27*) that the reappearance of the excretion phase occurs at the normal time. This would signify a normal renal transit time, which would be theoretically unfeasible after prolonged ischemia.

Our findings are in accordance with the investigations of Pavel et al. (*30*), who described a dome-like curve after tubular damage, with a late maximum and slow excretion phase. They also experienced the same mode of recovery in rat experiments as well as in humans, and were able, with the renogram and clearances, to predict a favourable outcome if the posttraumatic renogram showed only a moderate decrease of the second phase. As we also found, a horizontal curve does not yield precise information.

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